METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF REMOGLIFLOZIN ETABONATE AND METFORMIN HYDROCHLORIDE IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple, rapid and accurate RP-HPLC method was developed for simultaneous estimation of Remogliflozin etabonate and Metformin hydrochloride in tablet dosage form. A Sheisedo C18 (4.6mm X 250mm, 5µm) column was used with a flow rate of 1 ml/min and detection wavelength of 254nm. The Mobile phase used is Phosphate buffer 3.0 pH– Acetonitrile (30:70 %v/v). The retention time of Metformin hydrochloride and Remogliflozin etabonate was found to be 3.727 min and 5.007 min respectively. The linearity was observed in the concentration range of 12.5-75 µg/ml and 2.5-15 µg/ml for Metformin hydrochloride and Remogliflozin etabonate respectively. The proposed method was simple, rapid, accurate, precise and useful for the routine analysis.

Keyword:*Metformin hydrochloride, Remogliflozin etabonate, RP-HPLC, Validation.*

1. INTRODUCTION

Remogliflozin etabonate (RGE) is chemically 5 methyl-1-(propan-2-yl)-4-[4-(propan 2-yl oxy) benzyl] 1H pyrazol-3-yl-6-O(ethoxy carbonyl)-b-D glucopyranoside hemihydrate. [1] It is a SGLT-2 inhibitor. Remogliflozin etabonate is prodrug of remogliflozin. It inhibits the sodium glucose transport protein (SGLT2), which is responsible for glucose reabsorption in the kidney. By blocking this transport it causes blood glucose to be eliminated through the urine.

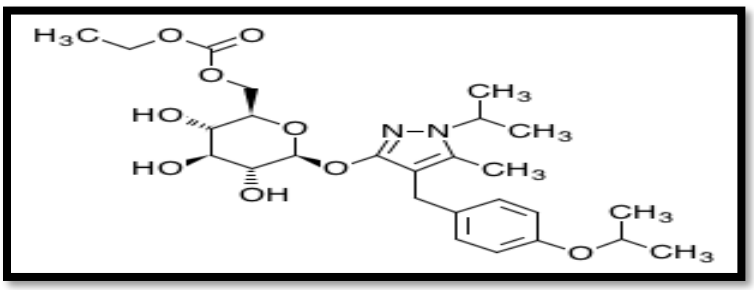

Fig 1: Structure of Remogliflozin etabonate

Metformin hydrochloride (MFH) is chemically 1,1-dimethylbiguanide hydrochloride. It is a Biguanide class of drug.2 Metformin is an antihyperglycemic agent.3 Metformin lowers hepatic glucose manufacturing, lowers intestinal absorption of glucose, and enhances insulin sensitivity by enhancing peripheral glucose uptake and utilization.

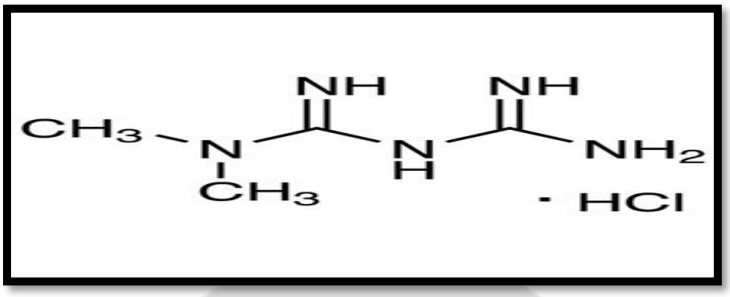

Fig 2: Structure of Metformin hydrochloride

2. METHOD AND MATERIALS

2.1 Instrumentation:

Analysis was performed on Shimadzu HPLC with UV-VIS detector Shimadzu SPD-20A VP used. The chromatographic system was analyzed by LC solution software. Chromatographic separation was performed on Sheisedo C_{18} (4.6mm X 250mm, 5µm) column. Analytical balance, Digital pH meter and Ultrasonicator also used in experiment.

2.2 Chemicals and Regents:

Metformin hydrochloride and Remogliflozin etabonate gift sample was obtained from Intas pharmaceuticals ltd and Glenmark pharmaceuticals ltd. HPLC grade solvents Methanol and Acetonitrile was obtained from Rankem. Remo-M 500 tablet containing Remogliflozin etabonate 100 mg and Metformin hydrochloride 500 mg was purchased from local market.

2.3 Chromatographic Conditions:

- Stationary Phase: Sheisedo C_{18} (4.6mm X 250mm, 5µm)
- Mobile Phase: Phosphate buffer 3.0 pH– Acetonitrile (30:70 %v/v)
- Flow rate: 1.0 ml/min
- Injection Volume: 20µl
- Run Time: 8 min
- Detection: 254 nm

2.4 Preparation of Solutions:

- Metformin hydrochloride

Stock Solution: - 100mg of Metformin hydrochloride was weighed accurately and transferred to a 100 ml of volumetric flask, dissolve in methanol. After sonicating volume was made up to mark with methanol to give a solution of 1000µg/ml.

Working Solution: - 50ml of standard solution transfer into 100ml volumetric flask and diluted up to mark with methanol to produce 500 µg/ml. Further dilution was made by diluting 2.5 ml from working solution to 100 ml with methanol to produce 12.5 µg/ml. Similarly 12.5-75 µg/ml wear made using methanol.

- Remogliflozin etabonate

Stock Solution: - 100mg of Remogliflozin etabonate was weighed accurately and transferred to a 100 ml of volumetric flask, dissolve in methanol. After sonicating volume was made up to mark with methanol to produce a solution of 1000µg/ml.

Working Solution: - 10ml of standard solution transfer into 100ml volumetric flask and diluted up to mark with methanol to give 100 µg/ml. Further dilution was made by diluting 2.5 ml from working solution to 100 ml with methanol to give 2.5 µg/ml. Similarly 2.5-15 µg/ml wear made using methanol.

3. RESULT AND DISCUSSION

RP-HPLC method developed for Metformin hydrochloride and Remogliflozin etabonate in combined dosage form. For the separation of Metformin hydrochloride and Remogliflozin etabonate Sheisedo C_{18} (4.6mm X 250mm, 5µm) was used with a flow rate of 1 ml/min. The detection wavelength of 254nm. The Mobile phase used is Phosphate buffer 3.0 pH– Acetonitrile (30:70 %v/v). The retention time of Metformin hydrochloride and Remogliflozin etabonate was found to be 3.727 min and 5.007 min respectively. The assay for Metformin hydrochloride and Remogliflozin etabonate was found to be 99.68 and 99.25 percentage.

3.1 Method validation:

Validation is defined as the "Documented proof that gives a high degree of assurance that the performed technique is in accurate with that of standard". The developed method was validated as per ICH guideline for system suitability, specificity, linearity, range, precision, accuracy, LOD, LOQ, robustness.

3.2 System suitability parameter:

System suitability tests are established on the idea that the equipment, electronics, analytical operations and samples constitute an integral system that can be assessed as a whole. System suitability testing offers assurance that the process will offer accurate and precise data for its planned use.

Table 1: System suitability parameter of MFH and RGE

System suitability parameter	Metformin hydrochloride	Remogliflozin etabonate
Retention time (min)	3.727	5.007
Resolution (R)	-	7.43
Tailing factor (T)	1.02	0.93
Theoretical plate number (N)	7206	8175

3.3 Linearity and Range:

It is the capacity to obtained results that is equivalent to its concentration. Range is the upper and lower concentration of an analyte for which it exhibit appropriate stage of accuracy and precision. Linearity is expressed in terms of correlation co-efficient of linear regression analysis. The linearity range for Metformin hydrochloride and Remogliflozin etabonate was found to be 12.5 – 75 µg/ml, 2.5 – 15 µg/ml respectively. Correlation co-efficient of Metformin hydrochloride and Remogliflozin etabonate was found to be 0.994 and 0.993 respectively.

Table 2: Linearity and range of MFH and RGE

Sr. no	Metformin hydrochloride			Remogliflozin etabonate		
	Conc. (µg/ml)	Mean area ± SD (n=3)	% RSD	Conc. (µg/ml)	Mean area ± SD (n=3)	% RSD
1	12.5	1581968.17 ± 76.04	0.004807	2.5	1380949.357 ± 195.13	0.01413
2	25	3082724.109 ± 248.17	0.00805	5	2693424.307 ± 121.33	0.00450
3	37.5	4532585.453 ± 158.01	0.003486	7.5	3958170.07 ± 249.09	0.00629
4	50	5974170.68 ± 245.64	0.004112	10	5215550.675 ± 311.63	0.00597
5	62.5	8036592.939 ± 234.57	0.002919	12.5	7077820.56 ± 298.07	0.00421
6	75	8957115.949 ± 199.50	0.002227	15	7857666.784 ± 286.39	0.00364

3.4 Precision:

It contain the closeness of agreement among collection of measurement obtain from more than one sampling of same homogenous sample under the prescribed situation. Precision perform in three stages: Repeatability, Interday and Intraday. Precision perform on three concentration level 75%, 100%, 125%. In repeatability each concentration mixture was repeated 3 times during the analysis. In intraday precision each concentration mixture was repeated 3 times on the same day (Morning, afternoon, evening). In interday precision each concentration mixturewas repeated 3 times on three back to back days. Mean area and % RSD wear found for each concentration.

Table 3: Precision of MFH and RGE

Drug	Conc. (µg/ml)	Repeatability		Intraday		Interday	
		Mean area ± SD (n=3)	% RSD	Mean area ± SD (n=3)	% RSD	Mean area ± SD (n=3)	% RSD
MFH	37.5	4531901.892 ± 672.80	0.01484	4532311.272 ± 1451.55	0.03202	4533976.775 ± 2321.58	0.05120
	50	5973718.107 ± 600.91	0.01005	5974270.308 ± 1061.91	0.01777	5974657.791 ± 2600.17	0.04352
	62.5	8035992.67 ± 540.38	0.00672	8035272.005 ± 1265.15	0.01574	8036346.93 ± 2845.80	0.03541
RGE	7.5	3957991.251 ± 680.75	0.017199633	3957539.712 ± 1696.65	0.04287	3955570.128 ± 2654.37	0.06710
	10	5215643.716 ± 580.44	0.01112893	5215328.494 ± 1395.86	0.02676	5214164.255 ± 2460.52	0.04718
	12.5	7076559.676 ± 611.20	0.008637101	7077226.418 ± 1136.59	0.01605	7077236.054 ± 2399.89	0.03391

3.5 Accuracy:

It is the closeness of the obtained results to its reference value. The % recovery experimentation was carrying out by the standard addition approach. Fixed quantities of sample mixture of Metformin hydrochloride and Remogliflozin etabonate and increasing quantity of its working solutions were spiked at 0%, 25%, 50% and 75%. Percentage recovery from the peak areas was estimate.

Table 4: Accuracy of MFH and RGE

Target conc. %	Spiked conc. %	Final conc. %	Metformin hydrochloride			Remogliflozin etabonate		
			Mean Area ± SD	% Recovery	% RSD	Mean Area ± SD	% Recovery	% RSD
50	0	50	3068517.92 ± 12157.8	99.53	0.39	2673041.81± 23267.1	99.24	0.87
50	0	50						
50	0	50						
50	25	75	4624765.93 ± 15435.3	98.37	0.33	4034580.70 ± 23144.2	98.59	0.57
50	25	75						
50	25	75						
50	50	100	6093859.56 ± 14435.8	98.13	0.23	5358707.04 ± 34651.8	99.71	0.64
50	50	100						
50	50	100						
50	75	125	7686834.72 ± 30976.8	101.89	0.40	6698658.30 ± 15553.6	101.73	0.23
50	75	125						
50	75	125						

3.6 LOD and LOQ:

Detection limit is the lowest amount of analyte that can be detected but not necessarily quantified. The LOD can be calculated as,

LOD = 3.3 × (SD / Slope)

LOD was found to be 0.0594 for Metformin hydrochloride and 0.01503 for Remogliflozin etabonate.

Quantitation limit is the lowest amount of analyte that can be detected as well as quantified. The LOQ may be calculated as,

LOQ = 10 × (SD/Slope)

LOQ was found to be 0.1802 for Metformin hydrochloride and 0.0455 for Remogliflozin etabonate.

3.7 Robustness:

The solution containing concentration of Metformin hydrochloride and Remogliflozin etabonate (50µg/ml and 10µg/ml) was analyzed at altered mobile phase, pH and flow rate. The peak area found for each solution was measured and %RSD was calculated.

Table 5: Robustness of MFH and RGE

Sr. no.	Metformin hydrochloride (50µg/ml)			Remogliflozin etabonate (10µg/ml)		
	pH	Flow rate	Mobile phase	pH	Flow rate	Mobile phase
	+ 0.2 units	+ 0.2 units	+ 2%	+ 0.2 units	+ 0.2 units	+ 2%
	– 0.2 units	– 0.2 units	– 2%	– 0.2 units	– 0.2 units	– 2%
1	5954023.26	5902158.43	5898743.16	5195431.85	5342985.35	5208771.25
	5984170.68	5931957.73	5898354.66	5225937.43	5203451.16	5185417.84
2	5982398.35	5915962.69	5996524.52	5219648.24	5307936.52	5256947.76
	5924357.23	5975731.54	5942755.49	5256572.37	5159843.64	5234275.66
3	5968423.37	5967436.14	5967319.76	5265740.73	5267545.62	5318938.26
	5956784.71	5902864.36	5986271.83	5284871.28	5257428.91	5307327.79
Mean	5968281.66	5928519.09	5954195.813	5226940.27	5306155.83	5261552.42
	5955104.21	5936851.21	5942460.66	5255793.69	5206907.9	5242340.43
SD	14188.0753	34402.6478	50194.39284	35717.1522	37751.3756	55227.6626
	29942.1163	36679.2324	43959.3265	29474.6403	48884.3844	61353.8048
%RSD	0.237	0.580	0.843	0.683	0.711	1.049
	0.502	0.617	0.739	0.560	0.938	1.170

3.8 Assay:

Applicability of developed method was tested by analyzing the commercially available Tablet formulation Remo M 500.

Table 6: Assay of MFH and RGE

Formulation (Tablet)	**Tablet amount (mg)**		**Amount Found (mg)**		**% Assay**	
	MFH	**RGE**	**MFH**	**RGE**	**MFH ± SD (n=3)**	**RGE ± SD (n=3)**
1	500	100	496.15	98.54	99.68 ± 0.52	99.25 ± 1.72
2	500	100	501.3	101.22		
3	500	100	497.8	97.98		

4. CONCLUSION

RP-HPLC method is developed and validated for the determination of Metformin hydrochloride and Remogliflozin etabonate in tablet dosage form. All the parameters are validated as per ICH guideline and appropriate for the regular quantitative analysis. The process is specific as there are not any interfering impurities and excipients. The proposed method is found to be precise, accurate and simple.

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